Evaluation of Chloropicrin as a Toxic Air Contaminant

Part B. Human Health Assessment

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December 10, 2009

Historical Background

- Chloropicrin was used as a warfare agent in WWI
- First used as a fumigant in flour mills in 1926
- NIOSH IDLH – 2 ppm
- ACGIH TWA-TLV – 0.1 ppm
- DPR placed chloropicrin into reevaluation based on air monitoring data with levels greater than TLV

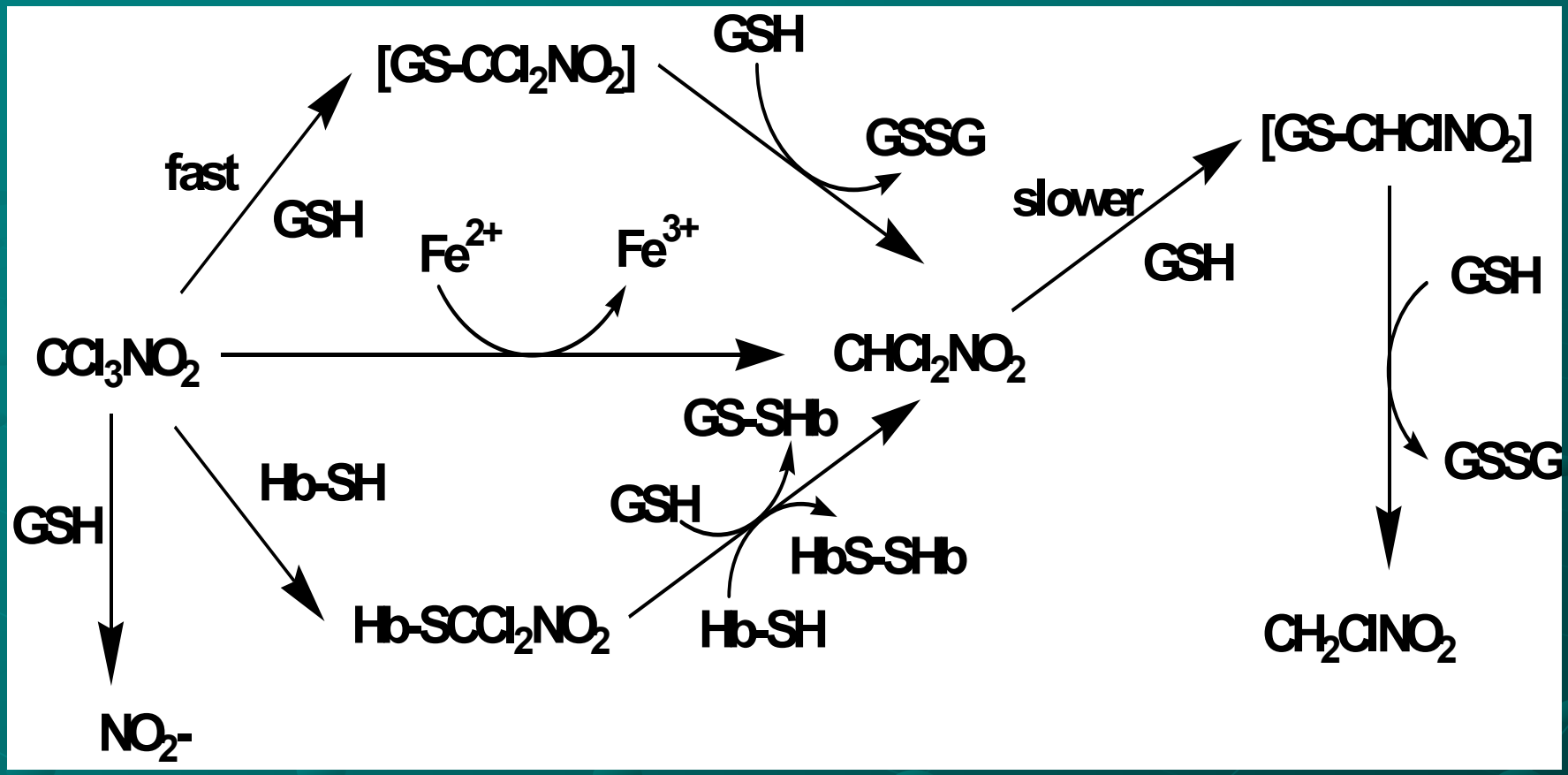

Figure 1 (p. 9). Proposed pathways for reaction of chloropicrin with glutathione and hemoglobin

Acute Toxicity – 1 Hour Exposure

Human sensory irritation study (Cain, 2004)

- Three phases
  - Phase 1 – Brief inhalation exposures (seconds)
  - Phase 2 – 20 minute exposure
  - Phase 3 – 1 hour exposure on 4 consecutive days

- DPR found this study acceptable
  - Conducted in accordance with GLP regulations and protocol approved by the IRB at U.C. San Diego
  - Protocol was reviewed by biostatistician to ensure there was sufficient statistical power
  - Approved by U.S. EPA's HSRB

Acute Toxicity – 1 Hour Exposure (cont.)

Human Sensory Irritation Study, Phase 3

- 32 Young adult subjects – 15 males and 17 females
- Subjects exposed to 0, 100 or 150 ppb for 1 hour on 4 consecutive days
- Rated eye, nose and throat irritation on scale of 0 to 3 every minute during their 1-hour exposures
  - No nasal or throat irritation reported
  - Eye irritation at 100 and 150 ppb

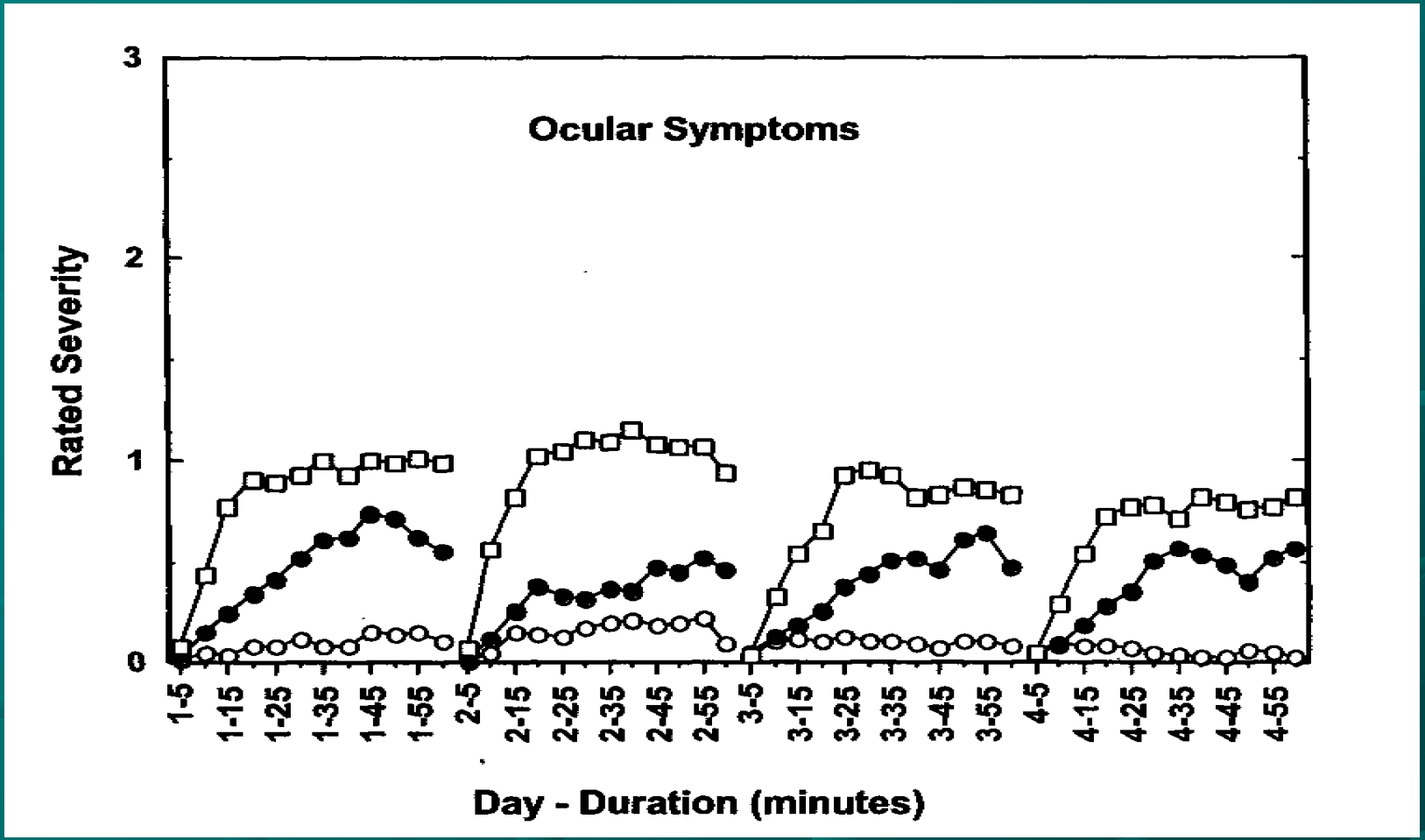

Figure 4 (p. 20). Average rated severity of ocular irritation by day of exposure of the human sensory irritation study for chloropicrin*

*(n = 32, males and females combined; blank = open circles, 100 ppb = solid circles; 150 = open squares)

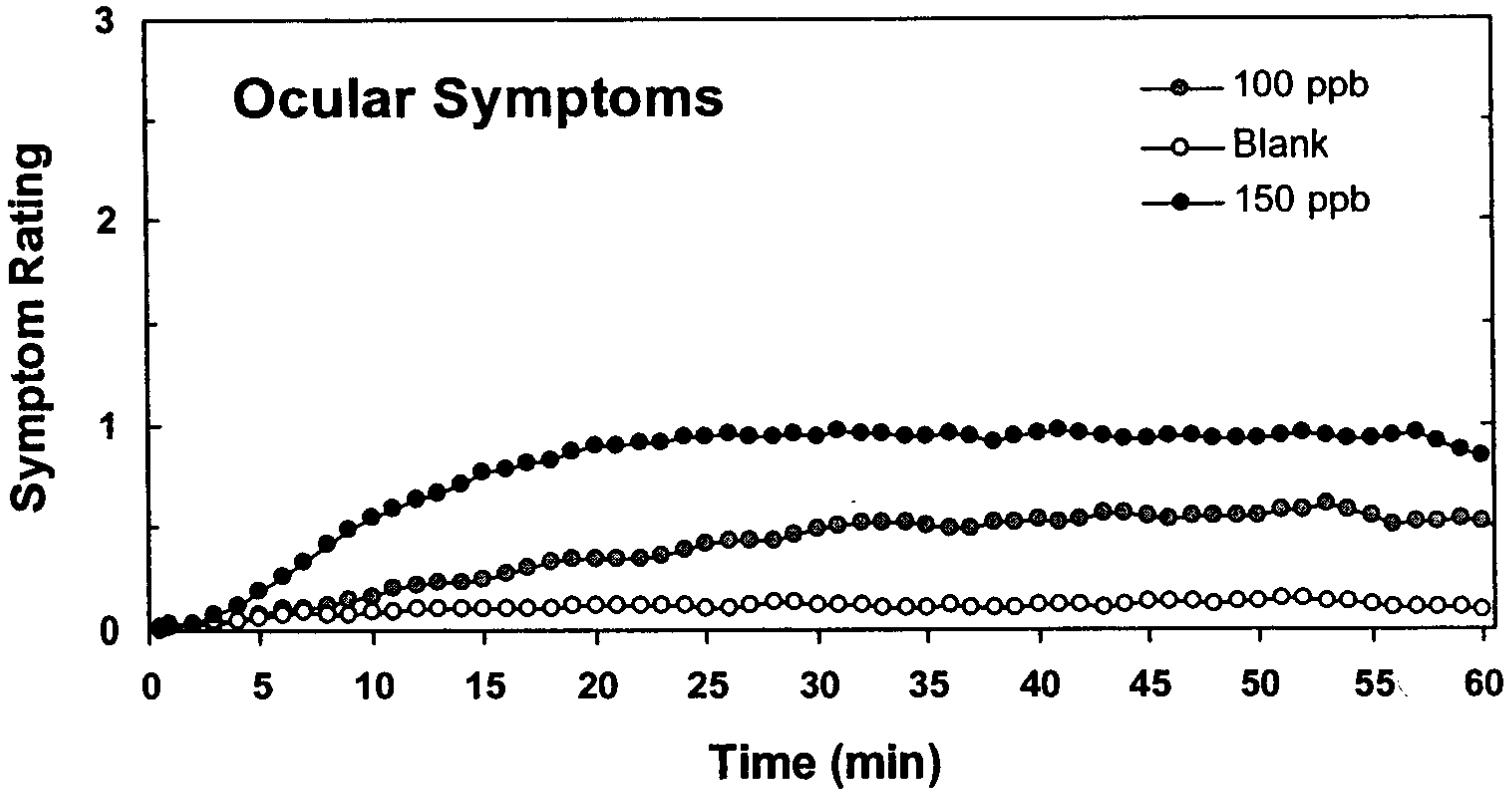

Figure 5 (abridged p. 21)**.** Average rated severity of ocular irritation during 1- hour exposures during phase 3 of the human sensory irritation study for chloropicrin*

* (n = 32, males and females combined).

Acute Toxicity - Human Sensory Irritation Study (cont.)

- Other respiratory variables evaluated in Phase 3
  - Lower respiratory variables unaffected
    - Nitric oxide (NO) concentration in expired pulmonary air
    - Pulmonary function (FVC and FEV_1)
  - Upper respiratory variables affected
    - Nasal air flow reduced at 150 ppb
    - Elevated NO concentration in expired nasal air at 100 and 150 ppb

Table 2 (p. 20). Ocular and Nasal Irritation in Human Subjects after 1-Hour Exposures for 4 Consecutive Days to Chloropicrin [a]

	Dose Level (ppb)		
	0	100	150
Ocular irritation Average score, overall[b]	0.10±0.19[c]	0.39±0.39	0.76±0.71
Average score, plateau[d]	0.12±0.22	0.54±0.51	0.90±0.86
Nasal Irritation Average increase in NO[e] in expired nasal air	1.6±15.6	12.0±11.9	12.7±16.6

a Cain, 2004.

b Average severity score reported for every minute of 1 hour exposure for all four days of exposure. Severity score ranged from 0 (no irritation) to 3 (severe – hard to tolerate and can interfere with activities of daily living)

c mean ± standard deviation n = 32, males and females combined since no significant gender differences

d Plateau period was defined as minutes 30 to 55 when the maximum scores were observed.

e The average difference in nitric oxide (NO) concentration (ppb) in expired nasal air before and after exposure for each individual for all four days of exposure.

Benchmark Dose Analysis for Human Study

- Threshold for identifying responders was estimated using the standard deviation in the control group
- Benchmark concentration at the 10% response level ($BMCL_{10}$) was used for eye irritation rather than the default of 5% because this effect was mild and reversible
- $BMCL_{10}$ for eye irritation was 26 ppb
- $BMCL_{05}$ for increased NO in nasal air was 44 ppb

Acute Toxicity – 8 and 24 Hour Exposures

Rabbit Developmental Toxicity Study

- Pregnant rabbits exposed to vapors 6 hrs/day from GDs 7-21

- Maternal effects observed in first few days of exposure were considered acute
  - Deaths
  - Red discolored lungs and pulmonary edema
  - Clinical signs of sensory and respiratory irritation
  - Reduced body weights and food consumption

- Acute NOEL = 0.4 ppm
  (8 hr HEC – 270 ppb; 24 hr HEC – 92 ppb)

Table 12 (abridged, p. 43). Acute Effects in Pregnant Rabbits Exposed to Chloropicrin Vapors During Gestation Days 7-20[a]

Endpoint	Dose Level (ppm)			
	0	0.4	1.2	2.0
Death	0 (0)[b]	0 (0)	1 (1)	8 (2)
Labored breathing	0 (0)	0 (0)	0 (1)	1 (2)
Excessive lacrimation	0 (0)	0 (0)	0 (1)	1 (2)
Nasal Discharge	0 (1)	0 (3)	7 (10)	1 (10)
Red discolored lungs	0 (0)	0 (0)	1 (2)	8 (2)
Edema in lungs	0 (0)	0 (0)	0 (1)	5 (2)
Body weight gain (g) GDs 7-13	-20 ±89	15 ±65	-243 ±165**	-407 ±194**
Food consumption (g) GDs 7-13	145 ±24	145 ±25	74 ±29**	32 ±28**

a York, 1993.

b Incidence outside and inside parentheses for GDs 7-11 and GDs 12-20, respectively.

Acute Toxicity – 8 and 24 Hour Exposures

Rabbit Developmental Toxicity Study (cont.)

- 1-hr RfC for ↑ NO in nasal air = 1.5 ppb for children, if additional uncertainty factor of 3 applied for children
- 8-hr RfC from rabbit study = 0.9 ppb for children applying an additional uncertainty factor of 3 for children
- Therefore, the 8-hr RfC derived from the rabbit study is still more health protective than 1-hr RfC from human study based on ↑ NO in nasal air

Subchronic Toxicity

90-Day Inhalation Toxicity Studies with Rats and Mice

- Exposure for 6 hrs/day, 5 days/wk for 13 weeks
- Effects at 1.03 ppm and higher
  - Mortalities and clinical signs
  - Reduced body weights and food consumption
  - Increased lung weights and pathological lesions in nasal cavity and lungs
- Benchmark dose analysis performed to determine most sensitive endpoint
  - Default 5% response level used since frank effects

Tables 3 and 4 (abridged, p. 24-25). Respiratory Lesions in Mice Exposed to Chloropicrin Vapors for 90 Days[a]

Effect	Sex	Dose Level (ppm)			
		0	0.3	1.03	2.89
Nasal Cavity					
Epithelial Hyalin Inclusions	M	0/10	0/10	3/9	10/10**
	F	0/9	2/10	6/10*	8/10**
Rhinitis	M	0/10	1/10	1/9	10/10**
	F	1/9	0/10	4/10	9/10**
Lungs					
Alveolar Histiocytosis	M	2/10	1/10	5/9	9/10**
	F	1/9	2/10	8/10**	10/10**

a Chun and Kintigh, 1993

*,** Significantly different from controls at $p < 0.05$ and 0.01, respectively by Fisher's exact test

Tables 5 and 6 (abridged, p. 27-28). Respiratory Lesions in Rats Exposed to Chloropicrin Vapors for 90 Days[a]

Effect	Sex	Dose Level (ppm)			
		0	0.3	1.03	2.89
Nasal Cavity					
Rhinitis	M	2/10	2/10	4/10	10/10**
	F	1/10	1/10	7/10*	8/10**
Goblet Cell	M	7/10	7/10	8/10	9/10
Hyperplasia	F	0/10	6/10*	7/10**	5/10*
Lungs					
Peribronchial	M	0/10	0/10	3/10	8/10**
Muscle Hyperplasia	F	0/10	0/10	6/10*	7/10**
Bronchial	M	0/10	0/10	4/10	9/10**
Epithelial Hyperplasia	F	0/10	0/10	5/10*	7/10**

a Chun and Kintigh, 1993

*,** Significantly different from controls at $p < 0.05$ and 0.01, respectively by Fisher's exact test

Table 15 (abridged, p. 51). Benchmark Dose Analysis of the Most Sensitive Endpoints in Mouse and Rat Subchronic Inhalation Studies

Species	Endpoint	Sex	$BMCL_{05}$ (ppb)	HEC (ppb) Child/Adult
Mouse	Epithelial Hyalin Inclusions	M	360	200/413
		F	84	45/96
	Rhinitis	M	650	350/746
		F	210	110/241
	Alveolar Histiocytosis	M	140	76/161
		F	81	44/93
Rat	Rhinitis	M	320	93/196
		F	**120**	**34/73**
	Peribronchial Muscle Hyperplasia	M	220	64/135
		F	160	46/98
	Bronchial Epithelial Hyperplasia	M	200	58/122
		F	180	52/110

Chronic Toxicity

Chronic Inhalation Studies with Rats and Mice

- Exposed for 6 hrs/day, 5 days/wk for 78 weeks (mice) or 107 weeks (rats)
  - Effects in mice at 0.5 ppm and higher
    - Reduced body weights and food consumption
    - Pathological lesions in nasal cavity and lungs
  - Effects in rats at 0.5 ppm or higher
    - Clinical signs and reduced survival
    - Reduced body weights and increased lung weights
    - Rhinitis
- BMD analysis performed to determine the most sensitive endpoint

Tables 7 and 8 (abridged, p. 30-31). Respiratory Lesions in Mice Exposed to Chloropicrin Vapors for 78 Weeks[a]

Effect	Sex	Dose Level (ppm)			
		0	0.1	0.5	1.0
Nasal Cavity					
Epithelial Hyalin	M	3/50	6/50	7/50	16/50**
Inclusions	F	10/50	11/50	24/50**	37/50**
Rhinitis	M	6/50	7/50	17/50**	35/50**
	F	3/50	6/50	18/50**	32/50**
Lungs					
Alveolar	M	8/50	17/50	22/50	29/50*
Histiocytosis	F	14/50	14/40	19/50	35/50**
Bronchiectasis	M	0/50	3/50	28/50**	41/50**
	F	0/50	5/50	28/50**	44/50**

a Burleigh-Flyer *et* al., 1995

*,** Significantly different from controls at $p < 0.05$ and 0.01, respectively by Fisher's exact test.

Table 9 (abridged, p. 33). Respiratory Lesions in Rats Exposed to Chloropicrin Vapors for 107 Weeks[a]

Effect	Sex	Dose Level (ppm)			
		0	0.1	0.5	1.0
Nasal Cavity					
Rhinitis	M	20/50	24/50	21/50	35/50**
	F	18/50	17/50	26/50	23/50

a Burleigh-Flyer and Benson, 1995

** Significantly different from controls at $p < 0.05$ and 0.01, respectively by Fisher's exact test

Table 17 (abridged, p. 54). Benchmark Dose Analysis of the Most Sensitive Endpoints in Mouse and Rat Chronic Inhalation Studies

Species	Endpoint	Sex	$BMCL_{05}$ (ppb)	HEC (ppb) Child/Adult
Mouse	Rhinitis	M	130	70/149
		F	120	65/138
	Epithelial Hyalin Inclusions	M	290	160/333
		F	100	54/115
	Alveolar Histiocytosis	M	190	100/218
		F	150	82/172
	Bronchiectasis	M	50 (68)	27/57 (37/78)
		F	43* (59)	23/49 (32/68)
Rat	Rhinitis	M	230	67/141

* A BMR of 2.5% used for bronchiectasis instead of 5% due to adversity of endpoint. $BMCL_{05}$ shown in parentheses.

Weight of Evidence - Carcinogenicity

Genotoxicity Studies

- Numerous positive assays
  - 8 Reverse mutation assays with *Salmonella, usually with TA100* + S-9
  - *In vitro* Comet assay with TK6 cells
  - *In vitro* chromosomal aberrations assay with CHO cells
  - Sister chromatid exchange assay in human lymphocytes
- Significant negative assays
  - Forward mutation assay with mouse lymphoma cells
  - *In vitro* and *in vivo* micronucleus assays
  - *In vitro* chromosomal aberrations assay with human lymphocytes
- Based on these data, DPR concluded that a genotoxic mode of action for tumor formation may be possible

Weight of Evidence – Carcinogenicity (cont.)

Carcinogenicity Studies in Animals

- Inhalation Studies
  - Increase in the combined incidence of adenomas and carcinomas in the lungs of female mice
    - Significant trend ($p < 0.01$) and pairwise comparison ($p < 0.05$), when adjusted for survival
    - Dose-related increase in the multiplicity of the tumors
    - Slight shortening of time-to-tumor at high dose

Table 8 (abridged, p. 31). Possible Treatment-Related Neoplastic Lesions in the Lungs of Female Mice Exposed to Chloropicrin for 78 Weeks[a]

Lesion	Dose Level (ppm)			
	0	0.1	0.5	1.0
Lung Adenoma	13/48[+b] (27%)	9/48 (19%)	17/47 (36%)	19/49 (39%)
Carcinoma	0/48 (0%)	4/48 (8%)	3/47 (6%)	4/49 (8%)
Combined Adenoma and Carcinoma	13/48[++] (27%)	12/48 (25%)	20/47 (43%)	22/49 (45%)
Combined Adenoma and Carcinoma – Adjusted	13/42[++c] (31%)	12/41 (29%)	20/43 (46%)	22/41* (54%)

a Burleigh-Flyer *et al.*, 1995.

b Denominator is the number of animals that survived up to the day of the first tumor, 253 days.

c Animals at risk (denominator) determined by the Poly-3 trend test.

+,++ Significant trend based on the Armitage-Cochran trend test at $p < 0.05$ and 0.01, respectively, except for the adjusted incidence which was based on Poly-3 trend test.

* Significant at $p < 0.05$ using the pairwise comparison from the Poly-3 trend test.

Weight of Evidence – Carcinogenicity (cont.)

Carcinogenicity Studies in Animals (cont.)

Oral Studies

- Increase in mammary fibroadenomas of female rats
  - Significant by trend analysis ($p < 0.05$) and pairwise comparison ($p < 0.05$)

- DPR concluded that the evidence was sufficient to warrant a quantitative assessment of carcinogenicity

- Cancer potency estimated to be 2.3 $(mg/kg/day)^{-1}$ based on lung tumors in female mice

Table 19 (abridged, p. 59). DPR Critical Endpoints and Human Equivalent Concentrations for Chloropicrin

Exposure Scenario	HEC (ppb) Child/Adult	Effects at LOEL
Acute 1 hr	26/26	Ocular irritation in humans
Acute 8 hr 24 hr	270/580 92/190	Mortalities, nasal discharge, ↓ body wts. & food consumption, red discoloration of lungs of pregnant rabbits
Seasonal	35/73	Rhinitis in female rats
Chronic	23/49	Bronchiectasis in female mice
Lifetime	Potency = 2.3 $(mg/kg/day)^{-1}$	Lung tumors in female mice

TAC Listing Criteria

$$Margin\ of\ Exposure = \frac{HEC\ (ppb)}{Air\ Concentration\ (ppb)}$$

- Generally, a MOE > 100 is considered protective of human health based on the following assumptions:
  - Humans are 10 times more sensitive than animals
  - 10-fold variation in sensitivity in the human population
- To not list as TAC, MOE > 1,000
  - For sensory irritation MOE > 30
    - No interspecies UF needed
    - Intraspecies UF = 3 since toxicokinetic differences not expected with direct-acting mechanism of toxicity

TAC Listing Criteria

$$Risk = Potency\ (mg/kg/day)^{-1}\ X\ Exposure\ (mg/kg/day)$$

Carcinogenicity

- Risk < 10^{-6} is generally considered negligible
- To not list a TAC: Risk < 10^{-7}

Table 24 (abridged, p. 67). Worse Case Margins of Exposure for Bystanders Following Soil Fumigation with Chloropicrin

Exposure Duration	Margin of Exposure[a]		Target MOE for TAC Listing
	Children	Adult	
Acute – 1 hour Eye Irritation/human	0.0016	0.0016	30
Acute – 8 hour Deaths, lung path/rabbit	0.042	0.088	1,000
Acute – 24 hour Deaths, lung path/rabbit	0.084	0.18	1,000
Seasonal Rhinitis/rat	0.48	1.0	1,000
Annual Bronchiectasis/mice	0.76	1.6	1,000

a Margin of Exposure (MOE) = HEC / Air Concentration. HEC = Human Equivalent Concentration. Only MOEs for the application method with the highest worse case estimate is shown for each exposure duration.

Table 30 (abridged, p. 75). Margins of Exposure for Bystanders Following Soil Fumigation with Chloropicrin Using 50th Percentile

Exposure Duration	Margin of Exposure[b]		Target MOE for TAC Listing
	Children	Adults	
Acute – 1 hour Eye irritation/human	0.0060	0.0060	30
Acute – 8 hour Deaths, lung path./rabbits	0.15	0.32	1,000
Acute – 24 hour Deaths, lung path./rabbits	0.25	0.52	1,000

a Margin of Exposure (MOE) = HEC / Air Concentration. HEC = Human Equivalent Concentration. Only MOEs for the application method with the highest exposure estimate for each exposure duration is shown using the 50th percentile for application rate and field size.

Table 31 (abridged, p. 76). Margins of Exposure for Bystanders Following Soil Fumigation with Chloropicrin Using 50th Percentile and Half Mile from Field Edge[a]

Exposure Duration	Margin of Exposure[b]		Target MOE for TAC Listing
	Children	Adults	
Acute – 1 hour Eye irritation/human	0.024	0.024	30
Acute – 8 hour Deaths, lung path./rabbits	0.62	1.3	1,000
Acute – 24 hour Deaths, lung path./rabbits	2.5	5.2	1,000

a Margin of Exposure (MOE) = HEC / Air Concentration. HEC = Human Equivalent Concentration. Only MOEs for the application method with the highest exposure estimate for each exposure duration is shown using the 50th percentile for application rate and field size and assuming the bystander is standing ½ mile from the field edge.

Table 25 (abridged, p.68). Estimated Cancer Risks for Bystanders Exposed to Chloropicrin Following Soil Fumigation[a]

Application Method	Residential		Occupational	
	MLE	95% UB	MLE	95% UB
Bedded, tarped	3.4×10^{-2}	5.6×10^{-2}	2.0×10^{-2}	3.2×10^{-2}

a Target risk level for listing purposes is less than 1×10^{-7}.

Table 26 (abridged, p. 69). Margins of Exposure for Bystanders Following Structural Fumigation with Chloropicrin

Exposure Duration	Margin of Exposure[b]		Target MOE for TAC Listing
	Children	Adults	
Acute – 1 hour Eye irritation/human	0.72	0.72	30
Acute – 8 hour Deaths, lung path./rabbits	27	57	1,000
Acute – 24 hour Deaths, lung path./rabbits	12	26	1,000

a Margin of Exposure (MOE) = HEC / Air Concentration. HEC = Human Equivalent Concentration.

Table 27 (abridged, p. 69). Margins of Exposure for Indoor Air Following Structural Fumigation with Chloropicrin

Exposure Duration	Margin of Exposure[b]		Target MOE for TAC Listing
	Children	Adults	
Acute – 1 hour Eye irritation/human	0.057	0.057	30
Acute – 8 hour Deaths, lung path./rabbits	1.5	3.2	1,000
Acute – 24 hour Deaths, lung path./rabbits	0.54	1.1	1,000

a Margin of Exposure (MOE) = HEC / Air Concentration. HEC = Human Equivalent Concentration.

Table 28 (abridged, p. 70). Margins of Exposure for Bystanders Following Enclosed Space Fumigation with Chloropicrin

Exposure Duration	Margin of Exposure[b]		Target MOE for TAC Listing
	Children	Adults	
Acute – 1 hour Eye irritation/human	0.0011	0.0011	30
Acute – 8 hour Deaths, lung path./rabbits	0.040	0.085	1,000
Acute – 24 hour Deaths, lung path./rabbits	0.018	0.039	1,000
Annual Bronchiectasis/mice	1.1	2.4	1,000

a Margin of Exposure (MOE) = HEC / Air Concentration. HEC = Human Equivalent Concentration.

Estimated Cancer Risk for Bystanders Exposed to Chloropicrin Following Enclosed Space Fumigation

Exposure Scenario	MLE	95% UB
Enclosed Space Fumigation	7.4×10^{-2}	1.2×10^{-1}

Table 32 (abridged, p. 81). Comparison of DPR's and USEPA's Reference Concentrations

Exposure Duration	DPR RfC (ppb)		USEPA RfC (ppb)	
	Child	Adult	Residential	Occupational
Acute	8.7 UF=3[a]	8.7 UF=3	73 UF=1[b]	73 UF=1
Seasonal	0.35 UF=100[c]	0.73 UF=100	0.27 UF=30[d]	1.2 UF=30
Chronic	0.23 UF=100	0.49 UF=100	0.13 UF=30	0.50 UF=30

a UF = Uncertainty factor used to derive RfC. For eye irritation in humans, DPR assumed toxicokinetic variation = 1 and toxicodynamic variation = 3 for intraspecies variation.

b USEPA assumed both toxicokinetic and toxicodynamic variation for eye irritation in humans are 1.

c DPR did not use RGDR adjustment factor in calculating HEC from animal studies and instead used a default uncertainty factor of 10 for interspecies variation

d USEPA reduced the interspecies uncertainty factor to 3 since they used an RGDR adjustment in their HEC calculation.

Other Toxicity Issues Evaluated

- Prenatal and Postnatal Sensitivity
  - Fetal NOELs $\geq$ maternal NOELs in developmental toxicity studies in rats and rabbits
    - Fetal effects were nonspecific signs, possibly secondary to maternal toxicity
  - Pup NOEL $\geq$ parental NOEL in rat reproductive toxicity study
  - Neonates were not exposed directly from birth to PD28 and could be more sensitive due to the immaturity of their respiratory system, immune system and metabolic enzymes.
    - An additional uncertainty for children may be appropriate
- Endocrine effects
  - Some reproductive effects, but unclear if endocrine-related
    - Reduced number of implantation sites
    - Increased pre- and post-implantation losses
    - Late-term abortions

Conclusions

Soil fumigation

- All of the bystander MOEs are significantly less than the target MOEs
- The cancer risk estimates are significantly greater than the target risk level of 10^{-7}
- Clearly meets criteria for listing as a TAC

Structural fumigation

- All of the bystander MOEs are significantly less than their target MOEs
- MOEs for indoor air are also significantly less than their target MOEs
- Clearly meets criteria for listing as a TAC

Enclosed space fumigation

- Bystander MOEs are significantly less than target MOEs
- The cancer risk estimates are significantly greater than the target risk level of 10^{-7}
- Clearly meets the criteria for listing as a TAC